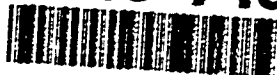


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TITLE: RESEARCH PROGRAM IN TROPICAL INFECTIOUS DISEASES

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CONTRACTING ORGANIZATION: Henry M. Jackson Foundation for the  
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BELIZE PORTION OF ANNUAL REPORT

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Research progressed on the causes of fevers of unknown origin, leishmaniasis and mosquito larval ecology. New initiatives included investigations of outbreaks of hepatitis and gastroenteritis. Manuscripts on gastroenteritis and hepatitis were prepared and submitted for presentation and publication. Suspected cases of tick-borne fever in an archaeological team were investigated. Five USUHS medical students conducted research and obtained clinical experience in Belize. Three Belizean physicians entered USUHS MPH and MTM&H graduate programs. ERC personnel attended two International scientific meetings, received formal training in three courses and presented research data at four medical meetings in Belize.

Tropical Infectious Diseases; Febrile Illness; Gastroenteritis;  
Leishmaniasis; Arboviruses; Mosquitoes; Hepatitis; RAI;  
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## FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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## INTRODUCTION

The objective of this grant is to establish, operate and manage research and teaching programs in overseas locations where the USUHS has established, or is in the process of establishing, bilateral research agreements. These centers are to serve as sites wherein research projects of programmatic interest to the USAMRDC in the field of tropical infectious diseases can be conducted by USAMRDC and USUHS personnel in collaboration with host national counterparts. The program also provides the opportunity to transfer technology to the host-country scientists and technicians through short-term and degree-granting programs. It also provides USUHS medical students, Master of Tropical Medicine and Hygiene students and Doctoral Candidates in Medical Parasitology and Vector Biology opportunities to obtain practical experience with tropical infectious diseases of the Western Hemisphere.

In Belize, the primary objective is to establish and maintain an Epidemiological Research Center (ERC) for infectious disease research and teaching in the Ministry of Health, Central Medical Laboratory (CML), Belize City.

The research objectives are:

1. Determine the etiology of acute febrile illnesses and jaundice; determine antibody prevalence against various arthropod-borne viruses (e.g., EEE, VEE, WEE, SLE, MAY, dengue, YF, VSV, etc), leptospirosis, hepatitis A, B, C, D and E, by age, sex, ethnic group and geographical location; maintain surveillance for epidemic disease due to arthropod viruses, especially dengue.
2. Determine prevalence of HIV, HTLV-1 and HTLV-2 infections in selected populations.
3. Determine patterns of drug resistance of *Neisseria gonorrhea* in various regions.
4. Determine patterns of malaria transmission; maintain surveillance for chloroquine-resistant *Plasmodium falciparum*.
5. Determine vectorial capacity of putative malaria vectors.
6. Address epidemiologic targets of opportunity (e.g., leishmaniasis in the Belize Defense Force (BDF); causes of febrile illnesses in British Forces Belize (BFB), U.S. Army Corps of Engineers, etc.).
7. Determine the effectiveness of repellents and fabric impregnants for protection of deployed troops from endemic vector-borne diseases.

8. Validate the remote sensing models developed for use in predicting temporal and spatial changes in malaria vector abundance in Mexico, in a second ecologically similar area.

### Background

Belize is located on the eastern coast of Central America at the base of the Yucatan Peninsula, surrounded on the west and north by Guatemala and Mexico and on the east by the Caribbean Sea. The jungle-covered Maya Mountains occupy the southwestern portion of the country, rising to 1122 feet at Victoria Peak; the remainder of the country is low, crop- or scrub-covered coastal plains. Belize was founded as a buccaneer settlement and entrepot. Today, Belize is an English-speaking country, having gained its independence from Great Britain in 1981. The population is estimated at approximately 175,000 people, and is made up of a mixture of Mayans, Garifuna (Afro-Amerindian), Blacks, East Indians, Creole and Caucasians. About half of the population resides in the major city, Belize City, located on the Caribbean coast. The capital, Belmopan (population approximately 5,000) is inland and was built as a Federal District after a devastating hurricane in 1961 destroyed the previous capital, Belize City. There are several other smaller cities; i.e., Punta Gorda, Stann Creek, Hill Bank, Orange Walk, San Ignacio and Indian Church, scattered through the coastal plain.

Medical care is provided by a socialized medical system and is centered around local health clinics and district hospitals in the smaller cities and towns and a large central hospital in Belize City. Emergency cases (largely surgical) are brought to the Belize City Hospital for care, sometimes by air ambulance provided to the government by one of the Christian groups that has established missions throughout the country. The Belize City Hospital was built about 1930 and is a two-story building backed on the sea. It has about 200 beds, of which about 60% are dedicated to acute surgical patients. The hospital is divided into male, female and pediatric wards for both surgery and medicine. There are, in addition, neonatal and intensive care wards. A small chemistry, hematology and immunohematology laboratory used for acute diagnostic procedures is located in the hospital. The majority of diagnostic and public health laboratory procedures are performed at the Ministry of Health CML, located about 3 miles north of the city. Current laboratory capabilities include: malaria smears (approximately 29,000 per year), bacteriologic cultures, routine biochemistries, and HIV antibody testing using commercially available ELISA kits.

### Febrile Illnesses

Little is known about infectious diseases specific to Belize itself. Based upon limited information from Belize and other Central American countries, it may be inferred that in Belize,

tropical infectious diseases are common. Yellow fever has been known to occur in the Yucatan,<sup>1</sup> dengue and malaria are endemic in Belize,<sup>2</sup> and cutaneous leishmaniasis, in almost epidemic numbers, has been reported in British troops stationed in Belize.<sup>3</sup> Cutaneous and visceral leishmaniasis have been reported in nearby Honduras and Guatemala.<sup>4</sup> Leptospirosis and Venezuelan equine and St. Louis encephalitis have also been found in neighboring countries.<sup>5</sup> Enterically-transmitted non-A, non-B hepatitis has recently been identified in Mexico. The Statistics Department at the hospital reported yearly admissions over the past several years for enteric fever of 4 to 6 cases; jaundice, 60 cases; and fever of unknown cause, about 150 cases. A great deal needs to be done to determine the prevalence and the incidence of tropical infectious disease agents in Belize.

In the current project, cases of unexplained fever were selected by trained Belizean collaborators from patients over 12 years of age presenting at the Belize City, San Ignacio and Orange Walk Hospitals. Patients with sickle cell disease, meningitis, dysentery, or evidence of peritonitis, wound infection, pneumonia, tuberculosis or HIV infection were not included in the study. Thick and thin malaria films were prepared from a finger stick and examined. Patients positive for malaria were listed but not studied. Patients selected for the study were divided into two groups, those with and without jaundice. A clinical exam was performed and blood was obtained for diagnostic tests. Considerable effort was expended (not always successfully) to get convalescent sera on discharged febrile patients. Sera were analyzed for the presence of antibodies to various arthropod-borne viruses, leptospirosis and hepatitis.

### Leishmaniasis

Cutaneous leishmaniasis is a zoonotic disease transmitted to man by human-biting female phlebotomine sandflies. Many *Leishmania* strains belonging to at least four species are capable of causing human disease. The clinical manifestations of the infection are primarily species-dependent, but a number of poorly defined host factors may influence disease expression. Flagellated promastigote-stage leishmanial parasites develop in the gut of female sandflies and are transmitted to the vertebrate host during a blood meal. Promastigotes rapidly parasitize macrophages, convert to the intracellular amastigote stage, and multiply. In the absence of specific immunity, they circulate to the regional or systemic reticuloendothelial system and ultimately cause sores and other manifestations after an incubation period of 1-6 weeks or longer.

Cutaneous leishmaniasis occurs in China, India, the Mediterranean basin, Africa, and Central/South America, but recently, cases have also been reported in the southcentral United States. In the



Old World, leishmaniasis is caused primarily by infection with *L. tropica*; in the New World, the main species are *L. mexicana* and *L. braziliensis*. Over a dozen different strains and subspecies have been found to be capable of causing human disease.

In the New World, particularly, cutaneous leishmaniasis presents with skin lesions as a major manifestation, but regional lymphatic chains are frequently involved as well. Typically, erythematous macules appear at the inoculation sites up to months after infection, followed by papules. The papules subsequently become nodular and may ulcerate to form well-circumscribed ulcers with indurated margins and necrotic eschar-covered bases. In the absence of bacterial superinfection, which is unusual, the lesions are not painful. Satellite lesions may be found a small distance from the primary ulcers in otherwise normal-appearing skin. Some strains appear to cause a milder form of the disease, and the primary lesions may not ulcerate but instead present as nodules, papules, or eczematous plaques. Nontender nodules and inflammation can develop along draining lymphatics. Regional lymphadenopathy is common, and parasites can be isolated from these nodes. Metastatic spread in the New World is generally associated with *L. braziliensis* and may occur months or years after acquisition in up to 80% of infections. Infections caused by *L. mexicana*, *L. peruviana*, and some strains of *L. braziliensis* are thought to cause localized disease without mucous membrane involvement. Spontaneous healing appears to be quite common with *L. tropica*, *L. major* and *L. mexicana*, except when the ear (pinna) is involved.

*Leishmania mexicana* is primarily distributed in Mexico, Belize and Guatemala. It is commonly (in 40% of cases) associated with lesions limited to the pinna of the ear and classically occurs in those harvesting gum from chicle plants; hence the name, "chiclero's ulcer." Forest rodents are the natural reservoir for *L. mexicana*. *Leishmania braziliensis* is thought to occur in Guatemala, though its presence is not as well documented as that of *L. mexicana*. In the Old World, cutaneous leishmaniasis is typically ulcerative, and in many cases, heals spontaneously within several months. Spontaneous healing of New World lesions is much less predictable. Certain South and Central American species, notably *L. braziliensis*, may result in a slowly healing primary ulcer and the late development of a mutilating infection of the upper respiratory tract (mucocutaneous leishmaniasis or espundia). A rare form of leishmaniasis known as diffuse cutaneous leishmaniasis is characterized by massively parasitized nonulcerated nodules, specific cutaneous anergy, and a poor response to therapy. It occurs in both the New and Old Worlds. Serious illness with leishmaniasis has been reported as a manifestation of HIV infection.

The differential diagnosis of cutaneous leishmaniasis includes pyogenic bacterial, mycobacterial (*Mycobacterium marinum*), fungal (blastomycosis, sporotrichosis, histoplasmosis), and spirochetal

(yaws and syphilis) infections, plus lupus erythematosus, sarcoid and malignancy.

Following an outbreak within the BDF last year, cases in the BDF have been closely monitored.

### Gastroenteritis

Rotavirus is the major cause of severe diarrhea in children. Worldwide, it is estimated that there are 140 million cases, with 1 million deaths per year.<sup>6</sup> Approximately 50% of infants and young children hospitalized with diarrhea are infected with rotavirus.<sup>7</sup> Transmission is thought to be by the fecal-oral route. Seasonality is a poorly understood feature of rotaviral enteritis. In temperate climates, rotavirus is detected most frequently in the winter; however, in the tropics, it is detectable all year round, with seasonal trends that are less distinct.<sup>8</sup> LeBaron, *et al.*<sup>6</sup> and Glass<sup>9</sup> have reported that rotavirus incidence peaks (2 highest consecutive months) in Mexico, Belize and Guatemala during October-November.

### Hepatitis

In May 1991, concern that cholera may be spreading north through Central America prompted officials of the Belizean Ministry of Health to initiate an active search for possible cases. In the Cowpen area of southeast Belize, there were no reports of illness resembling cholera, but banana farm workers and local health officials were concerned about the large number of people who were or had been ill with hepatitis. In some cases, people had diagnosed themselves as having hepatitis, based upon the occurrence of jaundice. In others, the diagnosis had been made by local health professionals.

Cowpen residents considered the appearance of hepatitis to be an annual occurrence, associated with the dry season, and attributed the disease to the poor quality of the available water. However, many believed the 1991 dry season to be more severe and prolonged than usual, and the numbers of cases, particularly in young adults, to be greater than that observed in past years. Concern about hepatitis heightened when the death of a young, pregnant woman and her child were attributed to the disease.

The Belizean Ministry of Health responded to the apparent increase in hepatitis cases by requesting assistance from the Belize-United States Epidemiologic Research Center in Belize City. In collaboration with the Uniformed Services University of the Health Sciences and with laboratory support from the Walter Reed Army Institute of Research, a preliminary investigation was done in

May 1991 in an attempt to establish a diagnosis. The May investigation by the Belizean team established the presence of hepatitis in the Cowpen area and the need for further study. This was followed in June 1991 by an expanded effort to identify and define cases and to do a cross-sectional study of hepatitis markers and related variables in banana farm workers and their families.

### Mosquito Larval Ecology

As with most Central American countries, the numbers of malaria cases in Belize have increased dramatically in the last few years. Belize presently reports the highest annual parasite index of any Central American country.<sup>10</sup>

The vectorial roles of the different anopheline species present in Belize are poorly defined, even though several endemic species are known vectors in other geographical areas. At present, *Anopheles albimanus* is considered to be the primary malaria vector in Belize. Although there is evidence that this species is indeed a primary vector, the role of other species that occur on the coastal plain, or in the foothills and mountainous areas needs to be delineated. Improved understanding of the roles and biologies of the different vectors in different geographical areas should lead to improved targeting of malaria control interventions.

In 1990, we initiated a long-term program of research designed to delineate the species of anophelines present, their vectorial roles, and their ecology within Belize. We initiated this program by conducting faunistic surveys in northern Belize.

Surveys of the mosquito fauna and aquatic flora were conducted in Belize during the wet and dry season of 1990 and 1991. The primary objective was to collect taxonomic series of mosquito species found in Belize, with secondary emphasis on quantifying the presence/absence, abundance, and phytoecological relationships of anophelines within various aquatic habitats.

### Tick-borne fever

In April, Drs. Elon and Dianne Chase, U.S. archaeologists at Caracol, a Mayan Ruin in Mountain Pine Ridge, expressed concern over the number of tick bites being reported by the students and workers (one man, "tick man," had 134 ticks removed one day). Sera were collected from 47 individuals for analysis. Their concern was for Lyme disease, heretofore unreported in Belize, and identification of possible causes of a viral-like syndrome contracted earlier in the Spring.

## RESULTS AND PROGRESS

### Administrative

One ERC technologist participated in an Environmental Toxicology Workshop in Managua, Nicaragua, and discussed the workshop content at a meeting of MASICA (Medio Ambiente y Salud Para El Istmo Centro Americano) representatives in Belize City.

Two ERC technologists and two CML technicians attended an INCAP (Instituto de Nutricion de Centro America y Panama)-sponsored course entitled, "Laboratory Diagnosis of *Vibrio cholerae* 01 and *Shigella dysenteriae*." The training was in preparation for managing anticipated cases of cholera in Belize.

A film production crew "shot" high definition TV (HDTV) and NTSC video footage for presentation at the Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) in Boston, 2 December 1991. The crew consisted of representatives from Hillmann & Carr Productions, Washington, DC; REBO Studios, NYC; Dr. "Chester" Kalter, dermatologist, Potomac, MD; LTC Philip Lawyer, entomologist, Walter Reed Army Institute of Research (WRAIR); and Mr. Fred Duncan, research biologist, Uniformed Services University of the Health Sciences (USUHS), Bethesda, MD. The crew, supported by the ERC staff, "shot" HDTV footage of patients with leishmaniasis and NTSC footage of the epidemiologic investigation of hepatitis in Cowpen.

The ERC staff traveled to Cowpen, Catacamas Farm and Triobladen to distribute individual serologic results of the hepatitis investigation and to disseminate information on the safe use of water and the proper disposal of waste.

A new secretary was hired in November.

Colonel Krieg presented a lecture on infectious diseases endemic in Belize to a microbiology class at University College, Belize. A briefing on cholera and other waterborne diseases was presented to the staff of the Ramada Hotel.

A paper entitled, "An Outbreak of Leishmaniasis in the Belizean Defence Force," was presented at the 10th Medical and Dental Congress, Ramada Hotel, Belize City, Belize.

A biosafety workshop was presented to the CML staff and laboratory technicians from the district laboratories.

Colonel Krieg and one of the ERC technologists attended the annual meeting of the ASTMH in Boston, MA, 1-5 December 1991. The workshop, "Diseases of the Tropics: Telecommunications and Technology Transfer," was presented and received superb reviews.

The workshop demonstrated uses of telecommunications in medical technology transfer between U.S. academic centers and "Third World" institutions. Segments of high definition television footage, both live and transmitted via satellite and previously recorded, were presented. The subject areas included: 1) case presentations and clinical diagnosis of leishmaniasis, including a live uplink from WRAIR to Boston, 2) field epidemiologic investigation of cases of jaundice, 3) disaster relief operations, and 4) biospherics modelling and disease prediction.

The ERC had a booth at Health Week, a program of scientific presentations and displays by representatives of the various health-related activities in Belize. The mission and activities of the ERC were presented in a poster and with a slide show. Pamphlets on gastroenteritis were distributed to participants.

One of the ERC medical technologists and a CML technician attended the two-week course in Medical Parasitology at USUHS.

Two medical equipment repair technicians from USUHS spent two weeks repairing and performing preventive maintenance on equipment in the laboratory.

An entomology technician was hired to mount mosquito larval skins and to digitize maps and enter data into the Geographic Information System (GIS). The technician has received training from a Smithsonian Institute entomology technician on the fixation and mounting of mosquito larvae and field collection techniques.

Two abstracts and manuscripts, 1) "Outbreak of Rotaviral Gastroenteritis in Belize" and 2) "Initial Report of a Viral Hepatitis Investigation in Rural Belize," were submitted for presentation at the 37th Annual Commonwealth Caribbean Medical Research Council Meeting, 22-25 April 1992, Curacao, Netherlands Antilles and for publication in the West Indian Medical Journal. Initial draft manuscripts were attached to the last quarterly report.

The ERC had a poster in the U.S. Embassy exhibit at the National Agricultural Show in Belmopan. The ERC staff prepared a pamphlet on rotavirus which was well received by the show attendees.

Four USUHS medical students participated in ERC research projects and clinical work at Belize City Hospital. In August 1991, three Belizean physicians entered the USUHS MPH or MTM&H graduate program.

## Leishmaniasis

The incidence of leishmaniasis in the BDF has dropped significantly, with only 2 cases reported during the past 12 months. This apparently is a reflection of effective preventive medicine education and use of insect repellants. One of the two BDF cases did not respond to treatment with paromomycin ointment and developed lesions consistent with mucocutaneous leishmaniasis. The patient was medically evacuated to Walter Reed Army Medical Center for treatment with IV pentostam.

## Gastroenteritis

Rotavirus appeared to be responsible for a significant number of the cases seen in an outbreak of gastroenteritis last winter and spring, although undoubtedly there were other causes as well. In addition to one *Shigella flexneri* isolate, there were a number of cases that were negative for rotavirus, and no bacterial or parasitic pathogens were identified. It is possible that the stool specimens were obtained when the concentration of rotavirus was below the sensitivity of the ELISA kit, or that there was some other undetermined cause. In most cases of rotavirus infection, the viruses are not detectable after the 8th day of illness.<sup>10</sup> Some of the stool specimens were obtained 7 or more days after onset of illness.

Norwalk virus has also been associated with epidemics of gastroenteritis. Outbreaks usually occur in localized settings (ships, recreational areas, schools) and are caused by consuming contaminated water and/or uncooked foods prepared in an unsanitary manner.<sup>7</sup> Norwalk virus is a common cause of gastroenteritis in both children and adults, whereas rotavirus is most commonly seen in infants and children less than 4 years of age.<sup>10</sup> There are several other enteric viruses that produce gastroenteritis, but their medical significance has not been determined.<sup>7</sup>

The percentage of cases in individuals >4 years of age in the Belize outbreak is higher than would be expected in an outbreak due solely to rotavirus. The analysis of a greater number of stool specimens and the measurement of antibody titers for viral etiologies would further elucidate the etiology of cases.

The outbreak developed over a wide area of Belize. Although the team did not travel to the southern districts, the district medical officers in the south indicated that there had been no discernible increase in the number of cases of vomiting and diarrhea. The incidence peaked during the last week of February and the first week of March. Over 69% of the reported cases in the outbreak were <4 years of age, and there was no significant difference in the attack rate by sex. The age distribution was compatible with a rotavirus etiology.

It had been reported that the yearly outbreak of gastroenteritis in this area of the Americas should appear in the late fall, October-November.<sup>6</sup> In order to determine whether this outbreak was atypical with respect to season, the clinical records for the Matron Roberts Health Clinic (MRHD), Belize City, were reviewed back to January 1989. Reported cases of gastroenteritis were stratified by month, age and sex. The number of cases for each month was compared to the mean (53.6 cases/month) over the entire 28 months. Cases occurred more frequently than the mean between January-May 1989, February, March, May, July and August 1990, and between January-March 1991. In the months of September-December 1988 there were 15, 22, 31 and 35 cases, well below the mean number/month. The two consecutive months in each year with the highest numbers were January-February 1989, February-March 1990 and February-March 1991. Approximately 45% of the cases were in infants and children  $\leq 4$  years of age, and there was no significant difference in sex.

In Belize, outbreaks would appear to be seasonal, with a high incidence between January-May and a peak incidence during the months of January-March. This coincides with the rainfall pattern in Belize. The dry season in Belize begins in January (144.8 mm) and continues through February (68.6 mm), March (40.6 mm), April (61.0mm) and May (127.0 mm). These figures represent a 10-year average for Belize City. There is also a climatic anomaly (the "little dry" or "mauger" season) in August, when rainfall is below average, which coincides with the increased incidence of gastroenteritis seen in July and August 1990. The apparent correlation with the dry season has been reported for enteric fevers in developing countries.<sup>11</sup> This is apparently the result of the drying up of drinking water sources, with a concomitant increase in the concentration of waterborne organisms. These primary water sources may in themselves become the source of infection, or contaminated secondary water sources may be responsible. In the case of many of the villages involved in the outbreak in Belize, as the rainwater "vats" dry up, water is obtained from streams or rivers, which are often contaminated.

The mode of transmission of rotavirus is presumed to be fecal-oral, although it may also be by respiratory droplets.<sup>9</sup> It has been suspected that contaminated water or food may play a role in the transmission. Testing water samples by polymerase chain reaction amplification during periods of peak incidence may be productive.

This outbreak is compatible with a rotaviral gastroenteritis, although other etiologies may have been responsible for some cases. The review of the MRHC historical data supports the conclusion that the outbreak was seasonal, related to the dry season, and that the gastroenteritis season in Belize is January-March.

## Hepatitis

In this study, we found a high prevalence of people with prior exposure to HAV and HBV, some new cases due to HBV, and many other new cases without HAV or HBV markers. Additionally, we found a large proportion of people who are potentially infective for HBV. We could not completely assess the impact of HEV in this outbreak. Testing for HEV and its antibody is still in its infancy. We could not establish a serologic diagnosis for most of our collected cases of hepatitis. Therefore, these can be characterized as non-A, non-B hepatitis, and they could be due to HEV or some other unknown hepatitis virus.

Hepatitis A and E have a fecal-oral route of transmission. Prevention is strongly dependent upon a purified water supply system, good personal hygiene practices, and proper disposal of human waste. Our study would indicate that in Belize, hepatitis A is an illness of the very young, but that hepatitis E may be a new virus in the area, attacking the young adult population. Defining an attack rate for hepatitis E is complicated by the fact that the serologic testing for HEV is still in the developmental research phase.<sup>13</sup> The incidence of hepatitis E may be high but not detectable using current technology.

Hepatitis B and C are transmitted through direct contact with blood, blood products and possibly other body fluids in which the virus is present. Within the United States, hepatitis B has been acquired as a result of intravenous drug use with used needles, blood transfusions and sexual contact. HBV transmission has served as a model to approximate the transmission of the more deadly retroviruses, such as HIV. Prevention has been through passive/active vaccinations and behavior modification counselling. The individual who suffers the highest morbidity risk is one who is positive for hepatitis B surface antigen. The most infectious individual is also one who has hepatitis B surface antigen. Pathogenicity is greatly enhanced if the hepatitis delta particle is present.<sup>14,15</sup> HBV infection can lead to chronic active hepatitis, cirrhosis and hepatic cancer. Within Belize, there are only anecdotal reports about long-term morbidity. We do not know to what extent there are serious consequences of HBV infection such as hepatomas and death.

Our study reflects that HBV exposure has occurred in 73% of the population, and 16% are positive for HBsAg. Clinical jaundice was often due to new hepatitis B infection. There was no evidence that delta particle was present in any clinical case. A small number of people showed evidence of exposure to HCV. Infection by HCV appears to occur independently from B and is a frequent complication of transfusions.<sup>16</sup> The initial report of the Cowpen hepatitis outbreak has been submitted for presentation at the 37th



Annual Commonwealth Caribbean Medical Research Council Meeting, 22-25 April 1992, Curacao, Netherlands Antilles and for publication in the West Indian Medical Journal.

### Mosquito Larval Ecology

Of the four anopheline species present in the study area, *Anopheles albimanus* is the most common one. It occurs mostly on the coastal plain and is present in both the wet and dry season. *An. albimanus* may share habitat with *An. crucians*. *An. pseudopunctipennis* and *An. argyritarsis* were present during the dry season, and their distribution seems to be limited to the Karst and Mountain Pine Ridge regions.

### Tick-borne Fever

Preliminary results with ELISA tests showed 7 individuals positive for Lyme disease; however, two of these specimens were also positive for syphilis, a known cross-reactor. There are a number of antibodies to other infectious diseases which will cross-react. Preliminary results need to be confirmed with other diagnostic tests; i.e., indirect fluorescent antibody and polymerase chain reaction.

### CONCLUSIONS

Too few patients have been studied to date to draw inferences about major causes of FUO and jaundice; however, the prevalence of antibodies to a wide range of infectious disease agents is very high. Seroprevalence surveys are necessary to determine the geographic regions and populations where additional surveillance and prevention efforts are warranted.

Leishmaniasis is endemic throughout the forested areas of Belize. The incidence of leishmaniasis in BDF and BFB troops should continue to be monitored, and when necessary, interventions should be introduced. A comprehensive study of leishmaniasis should be started to define the epidemiology of the disease more completely in Belize.

Gastroenteritis in Belize apparently has a seasonal pattern unlike other countries in the region. The incidence of cases will be closely monitored throughout the year.

The incidence of hepatitis in the Stann Creek District is high. The behaviors and risk factors that cause this population to be particularly prone to hepatitis infection are a matter of speculation. We have knowledge of problems with water and food sanitation, but we know little about the health and cultural practices, beliefs and attitudes of these communities that relate

to the blood-borne spread of hepatitis. Risk factors must become known if we are to plan an effective intervention strategy. We have an indication that parenteral antimalarials, vitamins, antibiotics and fluids are used in the absence of physician supervision, and that dental practices occur outside a professional setting. Thoroughly documenting the threat of blood-borne disease transmission is a high priority.

Surveys of mosquito larval habitats have been conducted in the northern part of Belize in both the wet and dry seasons. All regions surveyed to date show the presence of diverse mosquito larval habitats that are temporally and spatially variable. Although we are seeing great ecological variability from one area to another, we are also beginning to see consistency in vector preferences for habitats and habitat-types. As more standardized data is compiled and incorporated within the GIS and augmented with remotely-sensed data, we hope it may be possible to predict increases in mosquito vector populations. A third faunistic survey will be conducted during the upcoming dry season to verify and enhance previously collected data.

The cause(s) of illness in the archaeological team has (have) not yet been identified and will remain the subject of increased surveillance.

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PAKISTAN PORTION OF ANNUAL REPORT

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Research continued on six current protocols of Viral Hepatitis, Acute Febrile Illness, Chronic Liver Disease, Malaria, Hepatitis on Pregnant Women, and Comparative Trial of Hepatitis B Vaccine. Protocols currently under a stop-work order effective October 14, 1991.

Tropical Infectious Diseases; Malaria; Hepatitis Febrile  
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## FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.



## ANNUAL REPORT FOR PERIOD ENDING 30 NOVEMBER, 1991

TITLE: Anopheline Vectors of Malaria in Urban and Rural Areas of Pakistan.

### INTRODUCTION

Malaria has been the leading public health problem in many countries of the world including Pakistan. The history of mankind reveals that malaria has played a major role in world events. The early works of Chinese and Hindu writers, as well as of Hippocrates and Plato, document the debilitating effects of the disease. As late as 1981, malaria was present in 104 countries, and 1 billion people were exposed to infection in the world. This disease infects 300 million people annually and may kill almost 5 million. Malaria occurs throughout much of Pakistan, but its annual incidence varies widely by location and time of the year. The rural population suffers from malaria during August to November which is the period of intensive cultivation in this country.

Five anopheline species have been incriminated as important vectors of malaria in specific ecological settings within Pakistan. These include the following:

1. Anopheles culicifacies (rural)
2. Anopheles stephensi (urban)
3. Anopheles superpictus (desert)
4. Anopheles fluviatilis (hilly region)
5. Anopheles maculatus (mountainous region) (1)

Intensive studies on the bionomics of Anopheles culicifacies have been conducted, and valuable observations have been reported on adult mobility, longevity, survivorship, activity cycles, gonotrophic rhythms, mating behaviour, resting behaviour, sampling methods and population size (2,3,4,5,6,7). The role of local anopheline species in the transmission of malaria needs to be quantified for various ecological settings in rural and urban environments of Pakistan. This can be done by identifying the extent of man-vector contact by season of the year, time and location; by determining sporozoite rates by species, location, collection method and season of the year, and by quantifying host preference of different anopheline species. Questions that require special attention include which species are transmitting malaria and under what condition does transmission

occur? Answers to these questions require specific and detailed information about host preference, sporozoite positivity rates under natural conditions, and the taxonomic status of local anopheline species.

## MATERIALS AND METHODS:

### 1. Study sites.

Six specific sites were selected: 3 in rural areas and 3 in urban settings of Rawalpindi and Islamabad. These locations were close to the breeding sites of mosquitoes (e.g., ponds, streams, etc.). The suggested study sites were:

Nur pur, Naril choak and Mohra (rural)  
Military farm, Dhamial camp and Mughal abad (urban)

### 2. Collections.

Early in the morning, adult resting collections were made at each site once a week for 12 months. At each site, the collections were made from cattle sheds and bed rooms. Time fixed for each collection was 30 minutes for each locality. Visits to these six sites were made three days per week. Mouth aspirators and mechanical aspirators were used for the collections. The captured mosquitoes were then transferred to paper cartons, and returned to the laboratory, killed by keeping in a freezer for a short time and identified using identification keys.

## RESULTS

A total of 5,828 mosquitoes (5,227 culicine, 601 anopheline) were collected during 1 December 90 to 30 November 91 from rural and urban locations in Rawalpindi and Islamabad. From collections of the previous year, a total of 4,466 mosquitoes were identified, out of which 3,511 (79.61%) were culicine and 955 (21.38%) were anopheline as shown in table 1. A relatively high density of culicine mosquitoes was recorded in the months of May, July and August. An almost equal density of both culicine and anophelines was recorded during the months of September, October and November. Of the 25 species of Anopheline mosquitoes reported from Pakistan, 12 species were found in the Rawalpindi and Islamabad areas (8). These were: An. culicifacies, An. fluviatilis, An. subpictus, An.

annularis, An. sergentii, An. maculatus, An. splendidus, An. willmorei, An. lindesayi, An. turkhudi, An. barianensis, and An. stephensi (Table 2).

Eight species of culicine mosquitoes were also collected including: Culex vagans, Culex infula, Culex bitaeniorhynchus, Culex fatigans, Culex univittatus, Culex fuscocephalas, Mansonia crassipes, and Ficalbia chamberlaini (Table 3).

## DISCUSSION

The anopheline species collected and identified round the year were Anopheline culicifacies, An. fluviatilis, An. stephensi, An. maculatus, An. subpictus, An. sergentii, An. annularis, An. splendidus, An. lindesayi, An. willmorei, An. barianensis and An. turkhudi.

For anopheline mosquitoes, Anopheles culicifacies was found in maximum numbers through out the year (67.74%). This was followed by An. fluviatilis and An. stephensi which were 17.80% and 4.39% of the total collected, respectively. The remaining species, An. culicifacies, An. fluviatilis and An. stephensi were found in low numbers throughout the year. A relatively high density of anopheline mosquitoes was recorded during the months of September, October, and November (44.63%, 53.58%, 40.18%) which is also the peak season of malaria in Pakistan. Although, most of the anopheline species were found in rural areas, Anopheles stephensi was found in both rural and urban environments.

Eight species of culicines were found in the Rawalpindi and Islamabad areas. These included Culex vagans, Ficalbia chamberlaini, Mansonia crassipes, Culex infula, Culex bitaeniorhynchus, Culex fatigans, Culex univittatus, Culex fuscocephalas. The data shows that Culex vagans and Ficalbia chamberlaini were found in maximum numbers (51.46%, 29.56%), while the population of Culex fuscocephalas was thin and sparse (0.25%). The high density of Culex mosquitoes was recorded for the months of May, July and August while the lowest density was found during the month of September. The density of mosquitoes depends upon the environmental conditions. In the rainy weather, the temperature decreases and humidity increases, therefore the density of mosquitoes increases in the rainy season. The genus Aedes was found quite sparsely during this period. Three species of Aedes were

collected during the month of October from rural areas: Aedes  
taeniorhynchus, Aedes albopictus and Aedes indicus.

Table 1. Percent collection of culicine and anopheline mosquitoes during 1990 by month.

| Month             | Culicine | %     | Anopheline | %     | Total |
|-------------------|----------|-------|------------|-------|-------|
| May               | 680      | 93.92 | 44         | 6.07  | 724   |
| Jun               | 336      | 97.10 | 10         | 2.89  | 346   |
| Jul               | 492      | 86.77 | 75         | 13.22 | 567   |
| Aug               | 795      | 92.22 | 67         | 7.77  | 862   |
| Sep               | 222      | 55.36 | 179        | 44.63 | 401   |
| Oct               | 298      | 46.41 | 344        | 53.58 | 642   |
| Nov               | 329      | 69.81 | 221        | 40.16 | 550   |
| Dec               | 359      | 95.98 | 15         | 4.0   | 374   |
| Grand total: 3511 |          |       | 955        |       | 4,466 |
| Culicine:         | 78.61%   |       |            |       |       |
| Anopheline:       | 21.38%   |       |            |       |       |

Table 2. Percent collection of anopheline mosquitoes during May-December 1990.

| Species                 | No. of mosquitoes | %     |
|-------------------------|-------------------|-------|
| <u>An. culicifacies</u> | 647               | 67.74 |
| <u>An. fluviatilis</u>  | 170               | 17.80 |
| <u>An. stephensi</u>    | 42                | 4.39  |
| <u>An. maculatus</u>    | 37                | 3.87  |
| <u>An. subpictus</u>    | 18                | 1.88  |
| <u>An. sergentii</u>    | 16                | 1.67  |
| <u>An. annularis</u>    | 14                | 1.46  |
| <u>An. splendidus</u>   | 3                 | 0.31  |
| <u>An. lindesayi</u>    | 3                 | 0.31  |
| <u>An. willmorei</u>    | 2                 | 0.20  |
| <u>An. barianensis</u>  | 2                 | 0.20  |
| <u>An. turkhudi</u>     | 1                 | 0.10  |

Grand total: 955

Male: 9%

Female: 91%

Table 3. Percent collection of culicine mosquitoes during May-December, 1990.

| Species                       | No. of mosquitoes | %     |
|-------------------------------|-------------------|-------|
| <u>Culex vagans</u>           | 1807              | 51.46 |
| <u>Eicambia chamberlaini</u>  | 1038              | 29.56 |
| <u>Mansonia crassipes</u>     | 424               | 12.07 |
| <u>Culex infula</u>           | 119               | 3.38  |
| <u>Culex bitaeniorhynchus</u> | 44                | 1.25  |
| <u>Culex fatigans</u>         | 41                | 1.16  |
| <u>Culex univittatus</u>      | 15                | 0.42  |
| <u>Culex fuscocephalus</u>    | 9                 | 0.25  |
| <u>Aedes taeniorhynchus</u>   | 9                 | 0.25  |
| <u>Aedes albopictus</u>       | 3                 | 0.08  |
| <u>Aedes indicus</u>          | 2                 | 0.05  |
| Grand total:                  | 3511              |       |
| Male:                         | 61%               |       |
| Female:                       | 39%               |       |

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2. Title: Identification of Ixodidae (Hard ticks) in Rawalpindi and Islamabad areas.

A total of 1,030 ticks were collected during 1 December 90 to 30 November 91 from different animals (goat, sheep, cow). Specimens were stored in 70% ethyl alcohol for later identification.

Annual Report For The Period Ending 30 Nov 1991

Title: Non-A, Non-B Hepatitis in Pregnant Women in Pakistan

INTRODUCTION

Hepatitis in which hepatitis A virus, hepatitis B virus, Delta agent, Cytomegalovirus, and Epstein Bar virus are not implicated is considered to be non-A, non-B hepatitis as no definite serological technique for the diagnosis of non A non B hepatitis are available. Thus NANB hepatitis, both enterically transmitted as well as parenterally transmitted, remains a diagnosis of exclusion.

AIMS AND OBJECTIVES:

The purpose of this study is to (a) identify a cohort of pregnant women with the diagnosis of HEV hepatitis, (b) to observe the clinical characteristics and course of disease in pregnancy and (c) to collect samples for etiological diagnosis of NANB hepatitis in pregnant women.

FOLLOW UP:

Patients are followed up for 12 months after enrollment.

METHOD AND MATERIAL:

Pregnant women with NANB hepatitis will be compared with normal pregnant women and pregnant women with other forms of viral hepatitis. In addition, data on nutritional, socio-economic and other demographic factors is also collected. Then, statistical analysis for the survivorship and variables

that influence disease outcome will be accomplished.

The subjects included in the study must meet the following criteria.

- 1) Be pregnant.
- 2) Clinically diagnosed as having acute viral hepatitis.
- 3) Be between the ages of 15-49 years.

The controls for the study are:-

- a) Non-pregnant females with diagnosis of HEV.
- b) Two non-sick pregnant females with normal liver function tests for each patient of fulminant hepatitis matched for the patient's age and her trimester of pregnancy.

The medical history was taken and physical examination of the patients enrolled in the study was carried out and recorded in the proformae. The serological testing included liver function tests and prothrombin time. Hepatitis profile will be done when the funds are available.

Fecal samples were collected. All these samples were collected within 72 hours of enrollment. Blood samples were also collected for period of 12 weeks at weekly intervals. After this, if the LFT's did not reach normal, patients were followed up every 2 months till they returned to normal at which point they were discharged from the study or if the abnormality persisted, were followed up for one calendar year.

Specimens for comparison from controls were collected

only once.

#### RESULTS:

In the study titled as NANB hepatitis in pregnant women in Pakistan, during the year 01 Dec 90 to 30 Nov 91 "32" patients were included. Of these 32 patients, 14 were the cases of viral hepatitis while 18 cases were included as controls. Five of these patients fulfilled the criteria of fulminant hepatitis. For these cases of fulminant hepatitis two controls each were taken.

Control non-pregnant females with clinical diagnosis of HEV inducted in the study were 18 in number. No case fatality was recorded in the pregnant females with HEV.

#### DISCUSSION:

The sera collected from patients with viral hepatitis and control is stored at 70 c and due to lack of funds hepatitis serology could not be done. The collection of the data and samples is still in progress.

Annual Report For Period Ending 30th. Nov. 91

Etiology of Acute Febrile Illness in Rawalpindi, Pakistan

Summary

This study was carried out to determine various causes of acute febrile illness in young men admitted in military hospital Rawalpindi. A total of 191 patients were studied over a period of 12 months ( Dec 1990 to Nov 1991). A complete medical history and physical examination was carried out and recorded in the proforma in each patient. Routine investigations like complete blood picture, urine analysis and blood films for malarial parasites were carried out. The blood samples for culture were taken in all the patients. Specialised investigations like Widal test and auto-antibody screening was done when necessary. Enteric fever was found to be the most common cause 21% followed by malaria (15.2)% and pneumonia (6.4%).

It is concluded from the study that the infections like enteric fever and malaria were still the common causes of acute febrile illness in our country. So the infectious diseases should be vigorously excluded before considering the auto- immune and neoplastic diseases as the etiology of pyrexia.

## Introduction

Fever has been recognised as cardinal manifestation of many diseases since ancient times as recorded in the Sumerian Cuneiform (1) and by ancient scholars like Hippocrates (2). Although Galileo in the 16th century and Santorio Santorio in the 17th century constructed devices to measure body temperature. An effective clinical thermometer was not developed until the beginning of the 18th century. Gabriel Daniel Fahrenheit (3) Wunderlich, in 1868 clearly established that abnormality of temperature was a sign of disease and normality of temp a sign of health (4). Since then, physicians have used fever as a reliable guide to the presence of disease and the responses of disease to therapy.

Early reports and even some more recent papers on the subject of persistent, unexplained fever were affected by haphazard collection of case material. Nonetheless some studies have demonstrated the changing and a wide spectrum of diseases that can cause fever. (5,6,7,8,9). The study of Petersdorf and Beeson, in 1961, avoided some shortcomings by selecting patients with fever before the diagnosis was reached and established specific criteria for fever (10).

In the recent years the practice of medicine has changed dramatically and even greater changes have occurred in the diagnostic methods. Microbiology, chemistry, hematology, immunology, radiology and nuclear medicine laboratories offer more varied and sophisticated services for the diagnosis of diseases and evaluation of AFI.

In the project titled as " Etiology of Acute Febrile Illness in Rawalpindi, Pakistan," one hundred ninety one cases were studied over a period of one year (Dec 90 to Nov 91) in order to obtain an upto date sample of adults with acute fever and to re-examine the diagnostic problems encountered in these patients. The Patients were selected from indoor wards of services hospital in Rawalpindi.

### Materials and Methods

Adult men aged 16 years or more were included in the study. The criteria for admission of the patients to the study were :

Temperature of 38 C or greater.

Duration of illness 14 days or less.

Routine investigations, mainly complete blood count, ESR and urine analysis revealing no cause of fever.

No history suggestive of intake of drugs that may cause fever.

For the purpose of study, various terms for diagnosis were defined as follows:-

1. The diagnosis of typhoid fever included the patients with clinical features of typhoid fever i.e typical pattern of fever, toxic looks, hepatosplenomegaly, absence of leucocytosis, positive Widal test and isolation of Salmonella organisms from blood, urine or stools.

Similarly, the paratyphoid fever included all the patients in whom S.paratyphi organisms have been isolated

from blood, urine or stools.

Enteric clinical included the patients with clinical features of enteric fever but no organisms could be isolated from blood, urine and stool.

Fever not yet diagnosed (Fever NYD) included the patients in whom no definite diagnosis could be made after vigorous investigations.

Widal test was defined as negative if repeated titres of antibodies were below 1:160, and considered positive if rising titre of 1/160 or above or a single titer of 1:320 or above were found.

#### Clinical Examination

A detailed clinical history was taken and a thorough physical examination was performed and recorded as a check list on a specially designed proforma. All the participants were required to sign an informed consent form. The body weight was recorded at the time of admission. Vital signs were recorded every eight hours. The treatment with antipyretics and antibiotics was not started until appropriate blood, urine and stool samples were collected for investigations. An emergency treatment was instituted when the clinical condition of the patient warranted immediate therapeutic intervention. The patients were examined daily for the development of new signs and symptoms. Further investigations were also carried out as indicated by the clinical features and results of previous investigations.



### LABORATORY INVESTIGATIONS

Following laboratory tests were carried out:-

- a. Complete blood picture and ESR on the day of admission and repeated when necessary.
- b. Routine urine analysis on the day of admission.
- c. Blood complete picture on the day of admission.
- d. Thick and thin blood films for malarial parasites on admission and repeated if necessary.
- e. Widal test if clinically indicated and repeated to show a rising titre.
- f. Biochemical profile on admission which included serum urea, creatinine, blood glucose and liver function tests.

### CULTURES

The brain heart infusion broth, prepared in the microbiology section of the PULSE, was used to collect the blood samples from the cases of acute febrile illness admitted in military hospital Rawalpindi. Primary cultures were carried out from this broth on blood agar, chocolate agar, MacConkey agar and thioglycolate broth. All cultures were incubated at 37C. The thioglycolate broth was incubated for upto 7 days and when turbid subcultures were done on blood agar, chocolate agar and MacConkey agar. Urine samples were inoculated on blood agar, MacConkey agar, bismuth sulphite agar and Salmonella Shigella agar, as well as on Selenite F broth and GN broth. After 48 hours incubation the

broths were subcultured on MacConkey agar, bismuth sulphite agar and Salmonella Shingella agar. Plates were checked daily for upto 6 days and enteric pathogens were identified with API 20 E system. (Analytab Products. Incorporated, Plainview, Newyork).

All bacterial isolates were tested for sensitivity to amikacin, ampicillin, carbencillin, cefazolin, chloromphenicol, pipericillin, tetracycline, ticarcillin, cefalothin and trimethoprim/sulfamethyoxzole by disc diffusion technique in accordance with the National Committee for clinical laboratory standards.

#### SEROLOGY

The standard Widal WHO plate assay with locally prepared antigen was used for measuring S.typhi somatic (O) and flagellar (H) agglutinins as well as somatic (O) agglutinins of Salmonella paratyphi 'A' and 'B' strains. After making serial doubling dilutions of the serum starting from 1/20 to 1/1280, 2 drops of the locally prepared respective antigen was added to each well and after mixing thoroughly Widal plate was incubated for four hours. After that the plates were kept overnight at 4C and then agglutination was noted to find out the titre of the antibodies against Salmonella typhi and Salmonella paratyphi A and B.

In cases where the antibody titre was higher than 1/1280 further serial dilutions were made to find out the exact titre of the antibodies.

When the cause of the fever remained undetermined attempts were made to repeat all previous tests. The sera of all the patients have been preserved at -70°C for further analysis at the PULSE and at USUHS Bethesda, Maryland, USA and they will be used when deemed necessary.

### RESULTS

Out of the 191 patients studied the commonest causes of AFI was enteric fever clinical (21%). Other common etiologies of AFI were typhoid fever (12.2%), malaria fever (15.2%) and pneumonia (6.4%). No definite diagnosis could be established in about 40% of the cases.

Pulmonary and extrapulmonary tuberculosis was observed in 4.3% of the cases. Two point two percent (2.2%) of the cases were suffering from mumps. Of these three had presented with mumps parotitis and one with orchitis.

Paratyphoid fever was observed in 1.7% of the patients which is not in conformity with the annual report submitted to HJF for 1990.(11), because in the previous year we had the facility of Dupont Isolator tube, which is more effective in the growth isolation of micro-organisms.

### Discussion

The frequency of etiological causes of acute febrile illness in cases admitted in MH / Rawalpindi during the year Dec, 90 to 30, Nov, 91 is shown in table-I. A variety of clinical conditions presented with fever, the majority of which were gastrointestinal and respiratory illnesses.

This study shows that gastrointestinal ailments like

enteric fever, typhoid fever and paratyphoid fever are the most common causes of acute febrile illness in the Rawalpindi. The prevalence of these diseases can be explained by the lack of sanitation, inadequate water supply, improper sewerage disposal, and unhygienic conditions in general. In most of the areas of Rawalpindi the supply of drinking water is intermittent. During closing hours there is negative pressure in the pipeline which helps in sucking the contaminated sewerage water into the pipelines used for drinking water.

Other common conditions causing acute febrile illness were respiratory in origin, e.g. URTI, Pneumonia, tuberculosis and asthmatic bronchitis. Malaria was the next common cause of acute febrile illness. This is important to note because malaria can be diagnosed in majority of cases by routine blood film examination. Therefore, in our part of the world, the blood films must be thoroughly screened for the malarial parasite in cases of acute febrile illness.

By the analysis of clinical histories, physical examination and routine laboratory investigations, it was possible to diagnose most of the conditions causing acute febrile illness. But, there were still a number of cases which remained undiagnosed after routine analysis. These include certain bacterial and viral infections, autoimmune diseases and malignant diseases. These conditions may be investigated by radiological examination of the chest and abdomen. Biopsy of liver, lymphnode, bone marrow is a

suspicious local lesion blood examination for L E cells, total eosinophil count, serum protein electrophoresis, rheumatoid and antinuclear factor in serum, biopsy of superficial arteries, skin, muscle and kidney.

Differentiation of the diseases presenting as acute febrile illness is not only of academic interest but also of practical value, as many of them respond to treatment and are curable while in some cases preventive measures may be quite useful.

Hence, there is a need to adopt more comprehensive and detailed investigation to find out the exact cause of acute febrile illness in this part of the world.

Table - 1.

| <u>DIAGNOSIS</u>    | <u>CASES</u>          | <u>PERCENTAGE</u>      |
|---------------------|-----------------------|------------------------|
| 1. ENTERIC CLINICAL | 40                    | 21                     |
| 2. TYPHOID          | 23                    | 12.2                   |
| 3. PARATYPHOID      | 3                     | 1.7                    |
| 4. MALARIA CLINICAL | 18                    | 9.4                    |
| 5. MALARIA          | 11                    | 5.8                    |
| 6. URTI             | 16                    | 8.5                    |
| 7. PNEUMONIA        | 12                    | 6.4                    |
| 8. ENTERITIS        | 11                    | 5.8                    |
| 9. TUBERCULOSIS     | 8                     | 4.3                    |
| PULMONARY           | 5                     | 2.6                    |
| LYMPHADENITIS       | 2                     | 1.2                    |
| MENINGITIS          | 1                     | 0.5                    |
| 10. MUMPS           | 4                     | 2.2                    |
| 11. BRONCHITIS      | 2                     | 1.1                    |
| 12. UTI CLINICAL    | 1                     | 0.5                    |
| 13. FEVER NYD       | 40                    | 21                     |
|                     | -----<br>191<br>----- | -----<br>100%<br>----- |

Annual Report For The Period Ending 30th Nov, 1991

Etiology and prognosis of acute viral hepatitis in  
Rawalpindi, Pakistan

Introduction

Hepatitis is one of the most common causes of admission in Military Hospital (MH) Rawalpindi, Pakistan. Initially thought to be largely caused by hepatitis A, the adult patients studied at the PULSE between 1984 and 1986 revealed that hepatitis A almost does not occur in adults. Twenty three percent (23%) cases of hepatitis were caused by hepatitis B virus and the rest 77% by non-A, non B agent(s) (1). The findings of Qureshi et al in 1984 were also similar (2). Out of their 50 patients 1 was caused hepatitis A, 11 by hepatitis B and 38 by hepatitis non-A, non-B agents (2). During one year period from 01 Dec 1989 to 30 Nov 1990 two hundred and fifty six (256) cases of acute hepatitis were enrolled in the study at PULSE, Rawalpindi. No case of hepatitis A was detected while hepatitis B was found in 53 (20.7%) and non A, non B hepatitis was found in 203 (79.3%) patients. Therefore hepatitis B and agents of non A, non B hepatitis are risks for those living in or visiting Pakistan (3).

Hepatitis C, the major etiology of hepatitis following receipt of blood products, is known to cause disease that often results in chronic hepatitis (4,5,6). However, most of

the cases of Non A, non B hepatitis seen at MH had no history of receipt of blood products and appeared to have no chronic sequelae of hepatitis. This was substantiated by the analysis of 45 sera of histologically proven patients of chronic liver disease in whom there was very low prevalence of antibody to hepatitis C virus as compared to hepatitis B virus (7).

A new etiologic agent of hepatitis termed as enterically transmitted non-A, non-B hepatitis or hepatitis E was being described in south east Asia during this time. Outbreaks of hepatitis caused by enterically transmitted non A, non B agent(s) occurred in New Delhi and Ahmedabad cities of India, Kashmir, Nepal, Soviet Union, Africa and Mexico (8,9,10). These outbreaks were characterized by exposure to fecally contaminated drinking water (11). Several outbreaks of non A, non B hepatitis in Pakistan military have also been associated with fecally contaminated water (12,13).

In order to determine the etiology of hepatitis in adult men admitted in MH, we began a prospective study of all cases of acute hepatitis. This report summarizes the findings of cases collected through Dec 1990 to Nov 1991.

### Materials and Methods

#### Patients

Men at least 16 years of age admitted in MH Rawalpindi with jaundice were asked to participate in the study. Informed consent was obtained, medical record was reviewed and the patients were interviewed with a standard questionnaire. Physical examination was performed and



recorded in the proformae.

#### Laboratory

The sera were divided into aliquots at the PULSE. An aliquot was stored at -70C and another to be examined by ELISA for hepatitis A and B seromarkers. was also stored at -70c because regents for the analysis were not available. Upto 30 Nov 90, commercially available serologic test kits were used to study the sera for antibody to hepatitis A (HAVAB-EIA), IgM anti HAV (HAVAB-M EIA), hepatitis B surface antigen (AUSZYME) and anti HBC (CORZYME) Abbott Diagnostics. But as the funds were not available after Nov 90 serologic analysis could not be performed during this year (1 Dec 90 to 30 Nov 91).

#### Results

Between Dec 1990 to Nov 1991, 294 patients were enrolled in the study.

Fig I shows the cases of acute hepatitis by month. Risk factors for hepatitis are shown in Table I. Out of 294 patients 18.03 % had contact with hepatitis patients and 8.84 % had previous history of jaundice, 15.64 % had blood transfusion, 5.78 % had undergone surgery, 15.31 % had injections or inoculations and 7.48% had dental treatment. Comparison of the risk factors in different types of hepatitis will be made after the results of serologic analysis for hepatitis A, hepatitis B and non-A, non-B are available, which could not be done because of the financial

restraint and non availability of the test kits.

### Discussion

Studies on the etiology of hepatitis in men admitted in MH Rwp during the previous years indicated that viral hepatitis, both hepatitis B and Non-A, non-B, was the major cause of morbidity in troops near Rawalpindi, Pakistan. More than three-fourth of the cases of acute viral hepatitis were caused by non-A, non-B hepatitis and only about one-fourth cases were caused by hepatitis B. Only occasional case of hepatitis A was found during this period (1,2,3). For this year the etiologic frequency can be confirmed or otherwise when serologic analysis is performed on the sera collected during Dec 90 to Nov 91. The cases of non-A, non-B hepatitis may be primarily caused by hepatitis E (HEV: enterically transmitted non-A, non-B hepatitis) as a number of outbreaks of non-A, non-B hepatitis in Pakistan military have been associated with fecally contaminated water supplies (12). Viral particles in stools of Pakistani patients have been found to react with sera from HEV cases from Africa, India, Nepal and Mexico (Ticehurst J. Paper under publication). Therefore it appears that HEV may be a major cause of epidemic hepatitis in Pakistan. The putative viral agent of enterically transmitted non-A, non-B hepatitis (hepatitis E) has now been cloned and the serologic assay may become available for its diagnosis instead of just exclusion of hepatitis A and B alongwith epidemiologic characteristics

(14).

Hepatitis C (HCV) may also be an etiologic agent in some of the cases of non-A, non-B hepatitis in our patients. However, HCV does not appear to be a major problem in our region. We analyzed 45 sera for anti HCV in histologically proven cases of chronic liver disease and found that only 6 (13.3%) had this antibody in their sera (15). Moreover, only about 4% of cases of non-A, non-B hepatitis had a history of transfusion in the year prior to admission and had received injections (16). Although the serologic assay for the diagnosis of hepatitis C is available (17), there are certain problems in its use in acute cases of hepatitis C. The major problem is that the antibody is slow to develop and may not be detectable until upto one year after infection (16). For the in depth understanding of acute viral hepatitis following questions should be addressed in future.

- (1) Better diagnostic tests for hepatitis C & E.
- (2) Conduction of Trials for the protective roles of anti HCV and anti HEV by the administration of hyperimmune serum globulin prepared from convalescent sera of cases.
- (3) Development of vaccines for the protection against HCV and HEV infections.

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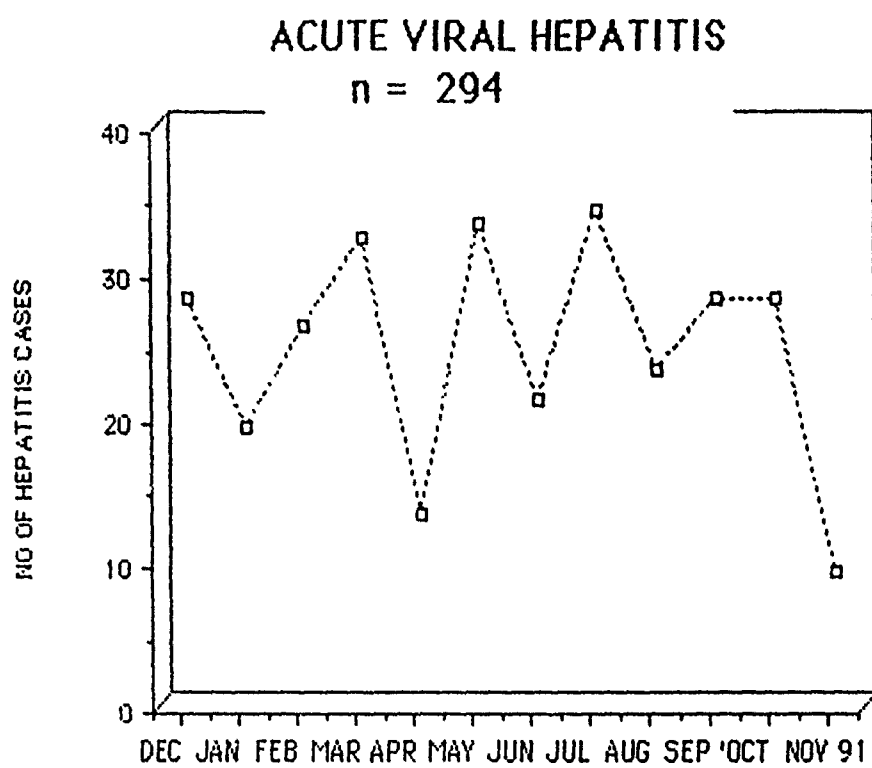


Table-1

|                              |         |
|------------------------------|---------|
| Contact with hepatitis case  | 18.03 % |
| Previous hepatitis           | 8.84 %  |
| Blood transfusion            | 15.64 % |
| Local or general surgery     | 5.78 %  |
| Injections within six months | 15.31 % |
| Dental surgery               | 7.48 %  |



Annual Report for The Period Ending 30th. Nov. 1991.

HEPATITIS C AS THE CAUSE OF CHRONIC LIVER DISEASE  
IN NORTHERN PAKISTAN

ABSTRACT.

The antibodies to hepatitis C virus (HCV) were tested in 45 cases of histologically confirmed chronic liver diseases. These cases included 12 patients (26.67%) of chronic hepatitis, 24 cases (53.3%) of cirrhosis and 9 patients (20%) of hepatocellular carcinoma. The anti-HCV was detected only in 6 patients (13.33%), out of which 2 patients (16.67%) were suffering from chronic hepatitis, 3 cases (12.5%) had cirrhosis and one patient (11.11%) was having hepatocellular carcinoma. There was no past history of blood transfusion in any of the 6 cases with anti-HCV. The patients of chronic diseases had a much higher prevalence of HBV infection reaching a positivity level of 77.78% in case of hepatocellular carcinoma. This study showed that in Northern Pakistan hepatitis C is not a common cause of chronic disease, whereas HBV infection plays an aetiological role in a much larger number of these cases. This observation is contrary to the reports from the western countries.

## INTRODUCTION

The aetiological agents of non-A , non-B hepatitis (NANB) have alluded the researchers for a long time since its discovery in early 1970's (1). However, the situation about NANB hepatitis has become more clear recently. The cloning of the virus of posttransfusion NANB hepatitis (hepatitis C) was a breakthrough (2) which lead to the development of serological assay for diagnosis of hepatitis C (3). Lately, the putative viral agent of enterically transmitted NANB hepatitis (hepatitis) has also been cloned and the serological assay may become available for its diagnosis in near future (4).

These recent developments have revealed that hepatitis C may be associated with blood transfusion but some cases of hepatitis C may be community acquired with no history of blood transfusion in the past (5). The hepatitis C accounts for 70-95% cases of posttransfusion hepatitis in the western countries (6). However, a substantial proportion of cases (90-95%) have no history of blood transfusion and this variety has been labelled as community acquired hepatitis (5). The hepatitis C has a high degree of chronicity with a substantial number of cases suffering from chronic hepatitis (41%), cirrhosis (20%) and hepatocellular carcinoma (5). The studies from various parts of the world have reported a considerable difference of prevalence of antibody to HCV ranging from 20% to 75% in cases of chronic liver diseases (7,8,9).

An attempt was made to assess the prevalence of Anti- HCV in histologically proven cases of chronic liver disease and to compare it

with exposure to hepatitis B virus (HBV) infection in cases of chronic liver disease in Northern areas of Pakistan.

### PATIENTS AND METHODS

This study was conducted in the Pathology Department, Army Medical College, Rawalpindi which receives laboratory specimens from most of the hospitals in the Rawalpindi/Islamabad area where patients are admitted from all over Northern Pakistan.

In cases clinically suspected of having chronic liver disease, the needle liver biopsies were performed irrespective of age and sex. The liver tissue was fixed in 10% formal saline and embedded in paraffin. Multiple serial sections of 3-4  $\mu$ m thickness were cut and stained with H&E and special stains like Reticulin, PAS reaction and Von Gieson were also carried out. These stained sections of the liver were examined with light microscope. All those cases which had histologically confirmed chronic liver diseases, like chronic hepatitis, cirrhosis and hepatocellular carcinoma were included in this study.

In these patients a detailed history was taken and the relevant information was recorded on a proforma. Ten ml. of blood was collected in vacutainers from these patients and sera were separated by centrifugation and stored at -70°C until analyzed for antibodies to HCV and HBV.

For detection of HCV antibodies in sera, the Abbott HCV EIA kits were used which employ an *in vitro* quantitative enzyme immunoassay for detection of antibody to proteins expressed by C100-3 clone region

of the HCV genome. Briefly, the serum was diluted in a specimen diluent and incubated with a polystyrene bead coated with recombinant HCV C100-3 antigen. If the antibody was present in the serum sample, immunoglobulins in the patients sample were fixed to the coated bead. After washing the immunoglobulins bound to the solid phase were detected by incubating the bead-antigen-antibody complex with a solution containing human immunoglobulins. The O-phenylene diamine (OPD) solution containing hydrogen peroxide was used as coloring agent.

The serum specimen with absorbance values greater than or equal to the cut off value were considered initially reactive.

For detection of HBsAg, anti-HBc and IgM anti-HBc, the Abbott laboratory kits namely AUSZYME, CORZYME and CORZYME-M were used.

### RESULTS AND OBSERVATIONS

A total number of 45 cases of histologically confirmed chronic liver disease were studied. The histological examination revealed that out of these, 12 cases (26.67%) were suffering from chronic hepatitis, 24 cases (53.33%) had cirrhosis and 9 cases (20%) were having hepatocellular carcinoma. The seromarkers of hepatitis revealed that 2 patients (16.67%) suffering from chronic hepatitis, 3 cases (12.5%) with cirrhosis and one patient having hepatocellular carcinoma (11.11%) were positive for antibodies to HCV (table-1). Out of these 6 anti HCV positive cases of chronic liver diseases, one patient of cirrhosis was also a carrier of HBsAg and another patient with hepatocellular carcinoma revealed an evidence of past HBV infection (anti-HBc

positive) (table-11).

A significant observation was that none of these cases had any history of blood transfusion in the past. A much higher rate of previous exposure to HBV infection (anti-HBc) was observed in cases of chronic hepatitis (58.33 %), cirrhosis (45.82 %) and hepatocellular carcinoma (88.89 %) (table-1). However, none of these cases revealed any evidence of hepatitis B infection in the recent past (IgM anti-HBc).

### DISCUSSION

After the cloning of hepatitis C virus (HCV) and subsequent development of serological assay for its diagnosis, many studies have been reported in literature describing the prevalence of HCV infection in various population groups in different countries (7-10). The present study revealed a low prevalence of antibody to hepatitis C in cases of chronic liver diseases (13.33%) in the Northern part of Pakistan and none of these cases had any history of blood transfusion in the past. This prevalence of HCV infection in Northern parts of our country is closer to findings of a study carried out in Taiwan (7), in which a prevalence of hepatitis C infection was found in 21.3% cases of chronic active hepatitis, 33.3% patients of cirrhosis and 33.7% cases of hepatocellular carcinoma, with an overall prevalence of 29% HCV infection.

From Saudi Arabia, Fakunle and co-workers (10) reported a nearly similar prevalence of HCV infection in patients of cirrhosis (28.9%) and hepatocellular carcinoma (25%).

In both the studies from Taiwan and Saudi Arabia, it was also

observed that HBV infection , as evidenced by HBsAg carrier rate, was much higher as compared to HCV infection . Our observations are similar to these reports from Taiwan and Saudi Arabia because we also found an increased prevalence of HBV infection in cases of chronic liver diseases, reaching a very high level of 77.78% positivity in case of hepatocellular carcinoma. Similarly , a recent study from South Africa has revealed HCV positivity in 110 patients (28.05%) out of 380 cases of hepatocellular carcinoma but a much higher rate of HBV infection (8).

It appears that HCV infection plays an important aetiological role in a larger proportion of cases of chronic liver disease in the western countries as compared with oriental population. Bruix and associates (9) reported that 56% cases of cirrhosis and 75% patients of hepatocellular carcinoma had antibodies to HCV in Spanish population. Another study from Spain revealed that 62% cases of chronic hepatitis and cirrhosis which were positive for anti-HCV had a history of blood transfusion in the past (11). The experience of the Italian research workers was also similar who reported a positivity rate of anti-HCV in 74% cases of cirrhosis and 65% patients of hepatocellular carcinoma (12).

The hepatitis C accounts for a substantial proportion of the cases of acute and chronic liver diseases in the United States (5). According to an estimate of the Centre for Disease Control (CDC), about 150,000 cases of hepatitis C occur annually in the USA, out of which 7500 to 15,000 have a positive history of blood transfusions. About 5000 cases eventually develop chronic liver disease with disturbed biochemical hepatic profile and nearly 15,000 patients end up with

chronic active hepatitis and cirrhosis (5).

In conclusion, the patients of chronic liver disease in Northern Pakistan have much less prevalence of antibody to hepatitis C virus as compared to antibody to hepatitis B virus. A pertinent finding is that none of our cases of chronic liver disease due to hepatitis C had past history of blood transfusion. The hepatitis C is not a major cause of chronic liver disease in Northern Pakistan and the other developing countries in East Asia and Africa. However, the situation is quite different in the western countries where there is very low prevalence of hepatitis B infection and hepatitis C infection is the predominant aetiological factor in evolution of chronic liver disease.

#### ACKNOWLEDGMENT

We like to gratefully acknowledge the Regional Field Manager, Abbott Laboratories (Pakistan)Ltd.,Karachi for donating the kits for the serological assays of anti-HCV in this study.

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TABLE-1

PREVALENCE OF HEPATITIS-C IN PATIENTS OF CHRONIC LIVER DISEASE  
IN COMPARISON WITH HBV INFECTION.

| DISEASE                      | SERA     |           |           |            |             |
|------------------------------|----------|-----------|-----------|------------|-------------|
|                              | ANALYZED | Anti-HCV  | HBsAg     | anti-HBc   | IgManti-HBc |
| CHRONIC HEPATITIS.           | 12       | 2(16.67%) | 3(25%)    | 7(58.33%)  | 0 %         |
| CIRRHOSIS.                   | 24       | 3(12.50%) | 5(20.83%) | 11(45.82%) | 0 %         |
| HEPATOCELLULAR<br>CARCINOMA. | 09       | 1(11.11%) | 7(77.78%) | 8(88.89%)  | 0 %         |
| Total:                       | 45       | 06        | 15        | 26         | 0 %         |
|                              | (100%)   | (13.33%)  | (33.33%)  | (57.78%)   | 0 %         |

Table -11

DETAILED SEROLOGY OF PATIENTS POSITIVE FOR ANTI-HCV

| PATIENT<br>NO | DIAGNOSIS            | SEROMARKERS OF AVH |          |             |          |
|---------------|----------------------|--------------------|----------|-------------|----------|
|               |                      | HBsAg              | anti-HBc | IgManti-HBc | anti-HCV |
| 1.            | CHRONIC<br>HEPATITIS | -                  | -        | -           | +        |
| 2.            | CHRONIC<br>HEPATITIS | -                  | -        | -           | +        |
| 3.            | CIRRHOSIS            | -                  | -        | -           | +        |
| 4.            | CIRRHOSIS            | +                  | +        | -           | +        |
| 5.            | CIRRHOSIS            | -                  | -        | -           | +        |
| 6.            | H.C.C.               | -                  | +        | -           | +        |

ABBREVIATIONS: - AVH : Acute viral hepatitis.

- H.C.C : Hepatocellular carcinoma.

Annual Report For The Period Ending 30. Nov. 1991.

Comparative trial of low dose, intradermal Recombinant  
and plasma derived hepatitis B vaccines

Hepatitis B virus (HBV) is most versatile of the hepatotropic viruses and can produce (a) an asymptomatic carrier state, (b) acute hepatitis (c) chronic hepatitis (d) cirrhosis and (e) fulminant hepatitis with massive liver necrosis. There is a large body of evidence indicating that protracted infection with HBV plays a definite role in the genesis of hepatocellular carcinoma (1). This agent also collaborates with the defective delta hepatitis virus conferring on it the capacity to replicate and cause delta virus disease (2).

Infection with HBV is a global problem and an enormous effort is being made to develop vaccines to bring the morbidity, oncogenicity and mortality of this agent under control (3). Plasma derived hepatitis B vaccine was first used in 1975 and the first recombinant hepatitis B vaccine was licenced in 1986 (4). The duration of its effect is variable but on the average it is effective for about 5 years (4). Three 1 ml doses are required but the 3 ml vial is so costly that its widespread use as a prophylactic measure has not gained much popularity. It was, therefore, thought that a

smaller dose may reduce the cost and vaccination may become cost effective. The efficacy of low\_dose intradermal vaccines against rabies, Rift Valley fever and tick-borne encephalitis had been proved in trials (5), therefore similar trials with low-dose intradermal hepatitis B vaccine were also carried out by workers in the field. These studies showed that three 2 ug doses of plasma derived vaccine given intradermally produced levels of antibody to hepatitis B surface antigen (anti HBs) approaching those resulting from three 20 ug doses of the same vaccine given intramuscularly, with acceptable local reaction (6). The availability of recombinant-derived hepatitis B vaccine provided the opportunity to conduct a trial comparing the immunogenicity and reactogenicity of low-dose intradermal and full-dose intramuscular recombinant derived hepatitis B vaccine with low-dose, intradermal, plasma derived hepatitis B vaccine.

## Materials and Methods

### Vaccines

Plasma-derived (Heptavax B) and yeast\_derived (Recombivax HB) hepatitis B vaccines were used both manufactured by Merck Sharp & Dohme (MST; West point PA).

### Subjects

Volunteers were recruited among medical student at the Uniformed Services University of Health Sciences (USUHS), Bethesda, MD, and medical students and employees of the Army

Medical College (AMC), Rawalpindi, Pakistan.

### Study Design

Volunteers (153) whose sera were negative for anti HBs, anti HBc and HBsAg were enrolled in a prospective study and randomized to one of 3 vaccine groups: recombinant-derived 1 ug in 0.1 ml intradermally; plasma derived, 2 ug in 0.1 ml intradermally; or recombinant derived 10 ug in 1.0 ml intramuscularly. All participants received vaccine on days 0, 30 and 150. The intradermal vaccines were administered in a double-blind manner using a 27-gauge needle on a tuberculin syringe over the deltoid muscle, by a nurse at USUHS and a paramedical technician at AMC experienced in this technique. Intramuscular doses were given in the deltoid muscle.

Vaccinees were examined by a physician on days 1, 2, 4 and 7 after each immunization for erythema, induration, local swelling, pigmentation and nodules at the injection site and lymphadenopathy in the regional lymph nodes. They were questioned about pain, pruritis, interference with the movement of the arm and systemic symptoms including fever, chills, headaches, nausea, vomiting, diarrhoea, myalgia, arthralgia, urticaria, wheezing and lymphadenopathy. Serum was collected on days 30, 150, 200 and 300 and tested for anti HBs and anti HBc. Students were notified of their results. Vaccinees with anti HBs concentrations  $< 100$  m IU/ml after 3 doses were offered a booster dose of plasma-derived vaccine, 2 ug in 0.1 ml intradermally.

### Laboratory Methods.

Commercially available radioimmunoassay techniques were used to determine levels of anti HBc and anti HBs (CORAB and AUSAB, Abbott Diagnostics, Abbott Park-IL). Anti HBs values were expressed as milli-international units per milliliter and were determined by comparison with WHO known standards (7). The samples with values of  $> 150$  mIU/ml were diluted 1/10 and 1/100 and assayed again.

### Statistical Methods

Difference in numbers of students with local reactions and numbers of students with antibody levels  $\leq 9.9$  m IU/ml were determined by X<sup>2</sup>. Geometric mean concentrations were computed using a personal computer and program (stat view 512+; Brain Power, Calabassas, CA). Significance of differences between groups was calculated by non paired t test (two-tailed) using log-transformed values.

### Summary of the results

(Paper already published - J. Inf Dis 1990; 162:789-793). The peak geometric configurations of antibody to hepatitis 'B' surface antigen at day 200 were 1094, .387 and 43 mIU/ml, respectively in volunteers vaccinated with 10 ug/ml recombinant vaccine intramuscularly, 2 ug in 0.1 ml intradermally plasma derived and 1 ug in 0.1 ml intradermally recombinant vaccine. By day 360, these concentrations had

fallen to 346. 124 and 19 mIU/ml, respectively. Number of subjects with antibody  $\geq 10$  mIU/ml at day 200 was similar between 10 ug recombinant and 2 ug plasma derived groups (94% vs 90%), while only 78% of the 1 ug recombinant group had protective concentrations of antibodies. Erythema and induration occurred in most subjects in both intradermal groups while pain was prominent at the intramuscular site especially after the second dose. Thus, plasma-derived vaccine, 2 ug in 0.1 ml intradermally, appears to be an acceptable cost saving method for hepatitis B immunisation, while recombinant derived vaccine, 1 ug in 0.1 ml intradermally, produced less satisfactory results.



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### Further Sample Collections

From each volunteer 4 further serum samples were collected at yearly intervals at the beginning of 1989, 1990, 1991 and 1992. The number of samples collected each year collected is given below:

1989 =71

1990 =71

1991 Jan=71

1991 Oct=71

The sera collected have been stored at - 70 C for further analysis as regards the level of antibodies i.e. anti-HBc and anti-HBs as and when the funds are available.